LIPOSOMES FOR TREATMENT OF INFLAMMATORY DISEASES

Thesis

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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PUBLICATIONS AND PRESENTATIONS DURING CANDIDATURE

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Varsha Komalla, Mehra Haghi

The book entitled “Targeting chronic inflammatory lung diseases using advanced drug delivery systems.” commissioned by Elsevier

Manuscript under submission: The potential for phospholipids in treatment of airway inflammation: an unexplored solution

Varsha Komalla, Mehra Haghi, Meenu Mehta, Fatima Achi & Kamal Dua

Journal: Current molecular pharmacology

Manuscript accepted: A Phospholipid-Based Formulation for the Treatment of Airway Inflammation in Chronic Respiratory Diseases

Varsha Komalla; Venkata Sita Rama Raju Allam; Philip Chi Lip Kwok; Behjat Sheikholeslami; Louisa Owen; Adam Jaffe; Shafagh A Waters; Brian Gregory Oliver; Hui Chen; Mehra Haghi

Journal: European journal of pharmaceutics and biopharmaceutics

Manuscript under revision: Impact of a Cargo-less Liposomal Formulation on Dietary Obesity-Related Metabolic Disorders in Mice

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Oral Presentations

Quercetin and resolvin D1 liposomes as a novel therapy for steroid-resistant airway diseases, Drug Delivery Australia, Wollongong, October 2017.

Liposomal formulation of quercetin and resolvinD1 for treatment of steroid-resistant airway inflammation, TSANZ, Adelaide, March 2018.

Novel phospholipid based formulation to reduce airway inflammation, NAMe, Sydney, November 2019.

Poster Discussion

The Effect of Novel DOPC Liposome on Preventing High-Fat Diet-Induced Metabolic Disorders in Mice

Varsha Komalla, Yik Lung Chan, Gerard Li, Baoming Wang, Brian G Oliver, Hui Chen, Mehra Haghi

University of Technology Sydney 3 Minute Thesis (3MT) 2017

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LIST OF ABBREVIATIONS

PL: Phospholipids

COPD: Chronic obstructive pulmonary syndrome

CF: Cystic fibrosis

Th2: T Helper Cell Type 2

Th17: T Helper Cell Type 17

IL-4: Interleukin-4

IL-13: Interleukin-13

IL-5: Interleukin-5

IL-9: Interleukin-9

IL-17A: Interleukin-17A

IL-17E: Interleukin-17 E

IL-17F: Interleukin-17 F

IL-22: Interleukin-22

TNF-α: Tumor necrosis factor-alpha

IL-1β: Interleukin-1-beta

G-CSF: Granulocyte-colony stimulating factor

IL-6: Interleukin-6

CXCL1: C-X-C Motif Chemokine Ligand 1

Gro-α: growth-regulated oncogene-alpha

CXCL2: C-X-C Motif Chemokine Ligand 2

CXCL8: C-X-C Motif Chemokine Ligand 8

IL-8: Interleukin-8

(TGF-) β: Transforming growth factor-beta

LTB4: Leukotriene B4

IP-10: Interferon-gamma inducible protein

I-TAC: Interferon-inducible T-cell chemoattractant

Mig: monokine-induced by interferon-c

CFTR: CF transmembrane conductance regulator

FEV1: forced expiratory volume

IgE: Immunoglobulin E

CXCR2: CXC chemokine receptor 2

p38 MAPK: p38 mitogen-activated protein kinase

GPLs: glycerophospholipids

PE: phosphatidylethanolamine

PI: phosphatidylinositol

PG: phosphatidylglycerol

PS: phosphatidylserine

PC: phosphatidylcholine

SM: Sphingomylein

DOPC: 1,2-dioleoyl-sn-glycero-3-phosphocholine

US-FDA: United States Food and Drug Administration

RDS: Respiratory distress syndrome

LPC: lysophosphatidylcholine

IP3: inositol triphosphate

DAG: diacylglycerol

AA: arachidonic acid

HETEs: hydroxy-eicosatetraenoic acids

EETs: epoxyeicosatrienoic acids

LCAT: lecithin-cholesterol acyltransferase

MCP-1: macrophage chemoattractant protein 1

PAF: Platelet-activating factor, 1-0-alkyl-2-acetyl-sn-glycero-3-phosphocholine)

OxPL: oxidized phospholipids

Nrf2: nuclear factor erythroid 2-related factor 2

HO-1: heam oxygenase-1

PPARγ: peroxisome proliferator-activated receptor gamma

NFκB: nuclear factor kappa-light-chain-enhancer of activated B cells

TLR: Toll-like receptors

PAMPs: pathogen-associated molecular patterns

PRRs: pattern recognition receptors

PGE_2: Prostaglandin E_2

ICAM-1: Intercellular Adhesion Molecule 1

MPO: Myeloperoxidase

NAG: N-acetyl-glucosaminidase

SAPS: 1-stearoyl-2-arachidonoyl-sn-glycero-3-phospho-L-serine

HRV: Human rhinoviruses

CCL5: C-C Motif Chemokine Ligand 5

CXCL8: C-X-C Motif Chemokine Ligand 8

IFN-β: Interferon-beta

DOPG: Dioleoylphosphatidylglycerol

TPA: 12-O-tetradecanoylphorbol 13-acetate

POPG: Palmitoyl-oleoyl phosphatidylglycerol

RSV: Respiratory syncytial virus

CD14: Cluster of differentiation 14

COX-2: Cycloxygenase-2

LPS: Lipopolysaccharide

MMP-1: Matrix metalloproteinases

PPC: Polyene Phosphatidylcholine

NAPE: N-acylphosphatidylethanolamine

HUVECs: human umbilical vein endothelial cells

VSMCs: rat vascular smooth muscle cells

DLPC: Dilinoleoylphosphatidylcholine

iNOS: inducible nitric oxide synthase

DSPC: 1,2-distearoyl-sn-glycero-3-phosphocholine

ERK: Extracellular signal-regulated kinase

Actinobacillus pleuropneumoniae (App)

MPS: mononuclear phagocyte system

SSMM: Sterically stabilized mixed micelles

MM: Mixed micelles

PEG: Polyethylene Glycol

LUVs: Large unilamellar vesicles

SUVs: Small unilamellar vesicles

MLVs: Multi-lamellar vesicles

pMDIs: pressurized metered dose inhalers

CFCs: Chlorofluorocarbons

IPF: Idiopathic pulmonary fibrosis

DPI: dry powder inhalation

ACI: Anderson cascade impactor

FPF: Fine particle fraction

EPR: Enhanced Penetration and retention effect

RES: Reticuloendothelial system

CAR: coxsackievirus–adenovirus receptor

RGD: arginine-glycine-aspartic acid

CTD-ILDs: Collagen Tissue Disease–associated Interstitial Lung Fibrosis

BOS: Bronchiolitis Obliterans Syndrome

MBSA: maleylated bovine serum albumin

O-SAP: O-steroyl amylopectin

PA: phosphatidic acid

PI3P: phosphatidylinositol 3-phosphate

APL: Apoptotic body like liposomes

LHLN: 6-lauroxyhexyl lysinate

NTM: nontuberculous mycobacterial infection

DPPC: dipalmitoylphosphatidylcholine

ALIS: Amikacin liposome inhalation suspension

MAC: Mycobacterium avium complex

ATS/IDSA: American Thoracic Society / Infectious Diseases Society of America

GBT: guidelines-based therapy

CFU: colony formation units

NCFB: non-CF Bronchiectasis

ORBIT: Once-daily Respiratory Bronchiectasis Inhalation Treatment

CRD: Chronic respiratory disease

18:1 liss-rhod-PE: 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N- (lissamine rhodamine B sulfonyl) (ammonium salt)

HBSS: Hank's balanced salt solution

ELISA: Enzyme linked immuno sorbent assay

TGA: Thermogravimetric analysis

DSC: Differential scanning caloriemetry

MTT: (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide)

DAPI: 4′,6-diamidino-2-phenylindole

Flu-WGA: Fluorescent wheat germ agglutinin

PEEP: positive end-expiratory pressure

Rrs: Total Resistance

Ers: Total elastance

Crs: Compliance

Rn: Newtonian resistance

H: Tissue elastance

G: Tissue damping

AHR: Airway hyperresponsiveness

PC-PLC: Phosphatidylcholine-specific phospholipase C

P-chol: Phosphocholine

WAT: white adipose tissue

NEFAs: non-esterified fatty acids

SAA3: Serum amyloid A3

CDP-choline: cytidine 5′-diphosphocholine

HFD: High fat diet

CPT-1a: Carnitine palmitoyltransferase I

FASN: Fattyacid synthase

SREBP-1c: Sterol regulatory element-binding transcription factor 1

SLC2a2: Solute Carrier Family 2 Member 2

HOMA-IR: Homeostatic Model Assessment of Insulin Resistance

RT-PCR: Reverse transcription polymerase chain reaction

PBS: Phosphate buffer saline

TG: Triglycerides

FFA: Free fatty acids

PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

FOXO-1: Forkhead Box O1

DC: Deoxycholate

Abstract

Inflammation is the body's defense mechanism to harmful stimuli such as microbial infection, allergy, stress or injury. Local or systemic inflammation is a major hallmark of several chronic diseases including asthma, obesity, diabetes, cystic fibrosis, cancer, irritable bowel syndrome and psoriasis among many others. Current treatments for the majority of these diseases do not address the underlying inflammation and in those conditions where inflammation is currently treated, there are several areas of unmet need where inflammation is not resolved with current medications or several side effects occur as a result of the treatment.

Phospholipids (PLs) are amphiphilic molecules capable of forming self-assembling structures in water, making them suitable excipients in drug delivery systems such as liposomes, niosomes, solid lipid nanoparticles and phytosomes. Phosphatidylcholine (PC) is the most abundant PL and is a major component of biological membranes.

The biological activity of some natural PCs and their mixtures have been demonstrated in the literature and some PCs have shown to have immunomodulatory properties in inflammatory conditions such as ulcerative colitis, arthritis and multiple organ injury. In gastrointestinal inflammation, PCs administered orally have shown local therapeutic effects; however, reduction of systemic inflammation following the ingestion of PLs has not been demonstrated. In systemic inflammation, systemic administration of naturally occurring PCs has shown anti-inflammatory properties. Natural PCs are a heterogeneous mixture of components, therefore the immunomodulatory efficacy observed in the studies cannot be attributed to one single component of the mixture.

Hydrogenated natural PLs and synthetic PLs are identified in the “Inactive Ingredient Guide” by the food and drug administration (FDA). However, no study to date has investigated the therapeutic efficacy of pure synthetic PLs in form of a solution or a liposomal formulation for the treatment of inflammatory conditions. We report the first application of empty liposomes made up of synthetic PC as an anti-inflammatory treatment, in a murine model of airway inflammation and a murine model of systemic metabolic inflammation.

The 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and cholesterol containing liposomes (referred to as UTS-001) were prepared using the thin-film hydration method and characterized in terms of size, charge and polydispersity index. Stability studies were performed for over two months. Furthermore, UTS-001 was successfully freeze-dried using trehalose and characterized as per above for long-term storage.

Asthma is a complex, chronic inflammatory respiratory disease characterized by shedding of the epithelium, airway smooth muscle hypertrophy and hyperplasia, mucus overproduction, and airway inflammation. Airway inflammation in response to environmental pollutants causes activation of epithelial cells. The release of inflammatory mediators and the influx of inflammatory cells into the airways have been attributed to the pathophysiology of asthma.

In this thesis, we have demonstrated that UTS-001 was taken up by airway epithelial cells in a time-dependent manner via caveolae-mediated pathway. Furthermore, *in vitro*, in the culture of primary epithelial cells isolated from cystic fibrosis and asthmatic patients, UTS-001 treatment caused a significant reduction in interleukin-6 (IL-6) levels following stimulation with tumor necrosis factor-α (TNF-α). The immunomodulatory properties of UTS-001 were further investigated in an ovalbumin model of allergic

airway inflammation. Treatment with UTS-001 resulted in reduced airway hyperresponsiveness demonstrated by improved lung function measurement and decreased eosinophil number in bronchoalveolar lavage.

Obesity is commonly referred to as a systemic low-grade inflammation, exemplified by increased inflammatory macrophage number in metabolic tissues (e.g. liver and fat), as well as increased circulating inflammatory mediators. Inflammation is a well-accepted mechanism underlying weight gain, insulin resistance, hyperglycemia, and dyslipidemia in obesity. Efficacy of UTS-001 in reducing weight and systemic inflammation was investigated in a murine model of a high-fat diet. Administration of UTS-001 (along with consumption of the high-fat diet) resulted in a reduction of high-fat diet-induced inflammatory mediators such as TNF-α and peroxisome proliferator-activated receptor-gamma (PPAR-γ) in the liver. Retroperitoneal fat, liver weight and body weight were reduced significantly in obese mice following treatment with UTS-001. Macrophage numbers were reduced in fat and liver and intraperitoneal glucose tolerance test demonstrated an improved glucose tolerance following administration of UTS-001 in the high-fat diet-fed mice. Furthermore, UTS-001 caused an increase in thermogenic marker, uncoupling protein-1 (UCP-1) in the brown fat which increases non-shivering thermogenesis that enhances fat burning. Our studies demonstrated that UTS-001 has the potential to be used in the treatment of obesity and diabetes.

In summary, the studies presented in this thesis demonstrate a suitable pharmaceutical formulation of UTS-001 with anti-inflammatory properties with the potential to be used as a treatment in addressing local or systemic inflammatory conditions.